TOXIC EFFECTS OF A WHOLE-BODY INHALATION SARIN (GB) VAPOR EXPOSURE IN THE GOTTINGEN MINIPIG

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1. ABSTRACT / INTRODUCTION

Exposure to nerve agent vapors may result in a diverse array of clinical responses including threshold effects to lethality over a relatively small range of dosages. In order to assess the toxic hazards of such exposures and define chemical defense materiel requirements, it is essential to fill gaps in toxicological databases that define the physiological progression; from the first noticeable effect (miosis) to potentially fatal effects of inhalation exposure. Although there are numerous published works investigating the progression of toxic signs elicited by sarin (GB) exposures, both by accidental exposures and in research applications, never has there been a systematic whole-body inhalation study investigating the effects on multiple systems in real-time. While information gleamed from accidental inhalation exposures provides valuable insights on the short and long term sequalae of the exposure, by their nature they do not provide this information until after the subject has been removed from imminent danger, detoxified, and stabilized. In the past, the logistical problems encountered when performing whole-body inhalation experiments with nerve agents have severely limited the ability to collect data in real-time during the exposure. Therefore, the majority of work encompassing nerve agent studies and real-time data collection involves subcutaneous or intravenous injections as the delivery route. While the data collected from these studies is invaluable for assessing medical treatments and short and long-term effects from the agents, the time course of effects (from first evidence of agent in the systemic circulation, to onset of signs, to progression from mild to moderate to severe signs, to death) and compartmental distribution is vastly different. Additionally, and most importantly, they do not address the most likely route of exposure on the battlefield, i.e., inhalation.

The study described here examines the kinetics of GB vapor exposure dosage as it relates to systemic concentrations of agent (internal dose) and the pharmacodynamics/ time course of effects (from first evidence of agent in the systemic circulation, to onset of signs, to progression from mild to moderate to severe signs, to death) and compartmental distribution. The current study utilizes technological advances that allow the collection of electrocardiogram (ECG), electroencephalogram (EEG), electromyogram (EMG), pupil constriction and blood chemistry data in real-time during a whole body inhalation exposure to vapor GB.

This study is not intended to portray that the progression of toxic signs for all nerve agent vapor exposures will be the same. Rather, it is intended to provide a "global" look at the progression of toxic signs of exposure in one animal. This "global" look at the progression of nerve agent toxicity is the first study of its kind. It is intended to be the first in a series of studies with the ultimate goal of experimentally defining nerve agent exposure levels that can be considered thresholds between "no observable effects" and "measurable biological effects".

The pig was chosen as our model for studying the effects of whole body GB vapor exposures because of anatomical and physiological similarities to humans (see Information Resources for Swine in Biomedical Research (USDA, 2000) for a comprehensive review). Additionally, the pig provides a significant advantage over rodents due to their larger size and thus the availability of a larger blood volume available for analysis.

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2. METHODS

2.1 Animals

A 4-month old (12 kg) male Ellegaard Gottingen minipigs (Marshall Farms, NY) was used in this study. A silicone catheter (Bard access systems, 6.6 Fr.) was implanted in the right external jugular vein and a subcutaneous tunnel was made so there was access to the catheter from the back of the pig's neck. During nerve agent exposures, the catheter was maintained by a continuous i.v. infusion of lactated Ringers solution and blood samples were sequentially withdrawn periodically.

The pigs were secured for the experiments by placing them in a custom-designed (Lomir Biomedical, Inc., Malone, NY) canvas sling. The frame holding the sling was constructed of airtight stainless steel pipe and Swagelok™ fittings. The pig was maintained in the sling by 2 straps that secured over the pig's shoulders and hips. A muzzle harness was placed over the animal's snout, and secured both laterally and ventrally to the stainless-steel framing, and prevented the animals from moving their heads from side-to-side. This enabled us to maintain a consistent angle and distance from the infrared (IR) camera to the animal's eye. The harness was fitted so that it did not interfere with the animal's ability to open its' mouth to breath.

2.2 Nerve Agent Generation

Whole body exposures were conducted in a 1000-liter dynamic airflow inhalation chamber. The Rochester style chamber is constructed of stainless steel with Glass or Plexiglas windows on each of its six sides. The interior of the exposure chamber was maintained under negative pressure (0.50" H_2O), which was monitored with a calibrated magnehelix (Dwyer, Michigan City, IN). A thermoanemometer (Model 8565, Alnor, Skokie, IL) was used to monitor chamber airflow at the chamber outlet. Isopropyl methyl phosphonofluoridate (Sarin or GB) was used for all vapor exposures in this study. Chemical agent standard analytical reagent material (CASARM)-grade GB was verified (usually 98.3 + 0.48 wt. % pure as determined by quantitative ^{31}P-NMR) and stored in sealed ampoules containing nitrogen. Ampoules were opened as needed to prepare external standards or to be used as neat agent for vapor generation. All external standards for GB vapor quantification were prepared on a daily basis. Triethylphosphate (99.9% purity), obtained from Aldrich Chemicals, Milwaukee, WI, was used as the internal standard for the GB purity assays. The vapor generation system is located at the chamber inlet and is contained within a stainless steel glove box maintained under negative pressure. A gas-tight syringe, containing the test material, is secured into a variable rate, pulse-free syringe drive with the material delivered into a spray atomizer. The compressed air breaks the liquid into fine droplets, and facilitates vapor formation. Typically, the syringe was loaded with 2-4 ml of liquid nerve agent (CASARM grade).

2.3 Infrared Pupillography

A Sony CCD black and white video camera (model XC-ST50) equipped with (2) IR 100-candlepower spotlights was focused on the animal's left pupil for the duration of the nerve agent exposure. Sequential images of the eye, under very low-level light conditions, were digitally captured for analysis and calculation of pupil area at a later time. Disposable self-adhesive Ag/AgCl electrodes (Nicolet) were placed on the pig's head (Fz, Cz, Oz, A1 and A2), limbs (standard lead II ECG configuration) and back for monitoring of EEG, ECG and EMG, respectively, during the exposure. A respiratory belt (Biologic, Inc.) was placed around the chest of the animal. The leads from the electrodes and the respiratory belt were plugged into a portable Bio-logic headbox that was attached to the frame of the sling. A single wire from the headbox was passed through an access port in the side of the chamber to the external Bio-logic monitoring system (Cee-Graph, Netlink system). The jugular catheter was passed through a separate access port in the chamber wall. A Baseline blood sample (approximately 5 mls) was drawn through the jugular catheter. Infrared pupil images, EEG, ECG, EMG and respiratory data were collected for a minimum of 5 minutes (at a rate of 256 data points/second) before exposure to nerve agent. The pig was exposed to 5.35 mg/m^3 vapor GB for 10-minutes (Ct=53.5 mg.min/m^3). However, the pig remained in the exposure chambers for an additional 15 minutes for out-gassing. The pig was then removed from the chamber and blood samples, pupil images and electrophysiology signals were collected for an additional 15 minutes.

3. RESULTS

3.1 Pupil constriction

The basis of infrared pupillometry is that infrared light reflects off the retina back through the pupil, thereby producing an image of a bright pupil, with clearly defined borders. Successive captured images of the pig's pupil can then be analyzed for a reduction in pupil area and graphed as a function of time. A program designed in LabView was utilized to calculate the area of the bright pupil based on the equation for the area of an ellipse; area= $A*B*\pi$, where A is the horizontal radius and B is the vertical radius. The baseline and subsequent images were quantified as described above and the pupil areas were calculated, off-line, and graphed vs. time (figure 1). There was a baseline pupil fluctuation of ±5% in pupil area. Therefore the time at which there was a 10% reduction in pupil area, as compared to the baseline average, was considered as the time of onset of pupil constriction. The definition of miosis used in these studies was a decrease in pupil area to at or below 50% of the baseline mean. The onset of pupil constriction (10% pupil constriction) begins at approximately 4 minutes, reaches 50% constriction at 8.5 minutes and reaches maximum miosis by 12 minutes.

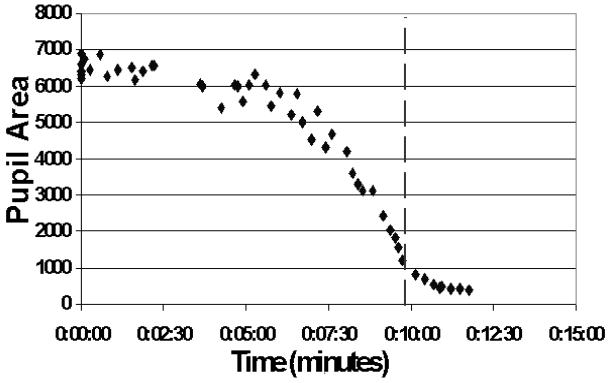


Figure 1. Pupil area vs. time of GB exposure. This figure is a graph of pupil area vs. time of GB exposure. The pig received a 10-minute exposure to 5.35 mg/m^3 vapor GB. Each point represents the pupil area calculated from 1 captured image. The points on the Y-axes were calculated from baseline images taken before the GB generation began. The end of the exposure time is indicated on the figure by the dashed vertical line. The onset of pupil constriction (10% pupil constriction) begins at approximately 4 minutes, reaches 50% constriction at 8.5 minutes and reaches maximum miosis by 12 minutes.

3.2 Systemic Agent

Whole blood fractions were drawn through the external jugular catheter before, during and after the GB exposure. After separation by centrifugation the red blood cells (RBCs) and plasma fractions were analyzed for regenerated GB (Jakubowski et al., 2002) and cholinesterase activity (Worek et al., 1999) and plotted vs time (figure 2).

REGENERATED GB: Regenerated GB is plotted vs time (left). Regenerated GB can be seen in the RBCs as early as 2 minutes after the onset of GB exposure and the amount rises steadily for the duration of the exposure. Regenerated GB is also measurable in the plasma (inset) but at a much lower concentration. In both the RBCs and plasma the regenerated GB plateaus at 30 minutes.

CHOLINESTERASE ACTIVITY: Cholinesterase activity is plotted vs. time (right). RBC acetylcholinesterase activity AChE has been corrected for hemoglobin content (Q value) and butylcholinesterase (BChE) activity has been corrected for total protein content (R value). AChE activity is reduced as early as 2 minutes after the onset of the GB exposure and has bottomed out by 8 minutes into the exposure. In contrast, BChE activity shows virtually no reduction in any blood sample.

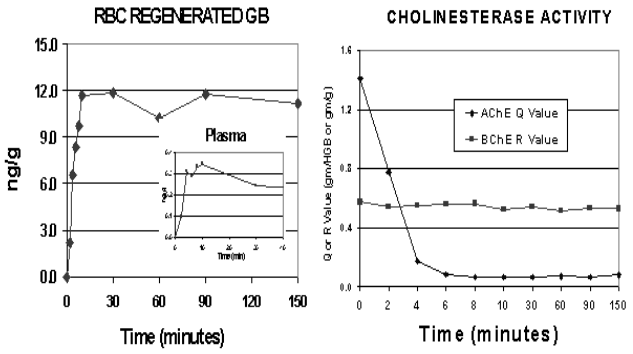


Figure 2

3.3 Respiratory changes

Tracings of the respiratory rate collected via the Biologic Inc. physiologic monitoring system using a respiratory belt are shown in figure 3. Approximately 20 seconds epochs are shown at various times during (every 2 minutes) and after the GB exposure (every 5 minutes). Over the first 6 minutes of the exposure the respiratory rate is relatively stable compared to the baseline. At approximately 8 minutes into the exposure there is a sudden increase in respiratory rate, which is shown graphically (upper right). However, at 10 minutes the respiratory rate begins to slow. From 15 to 25 minutes breaths become long, deep and irregular. These changes are evident in the graph of Area Under the Curve plotted vs. time. By 30 minutes, respirations begin to normalize but the rate is still elevated.

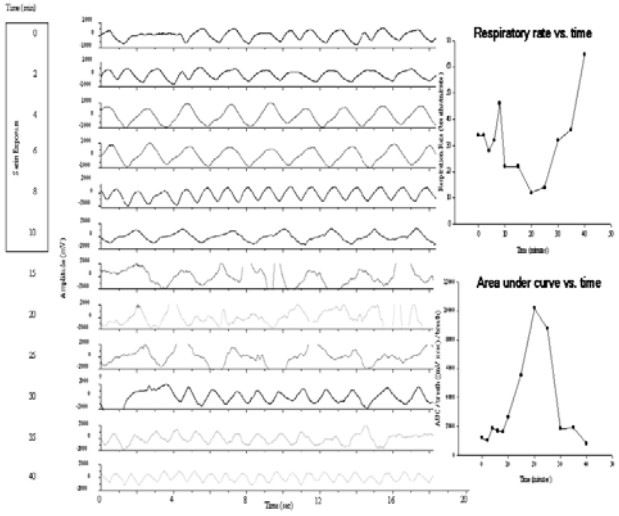
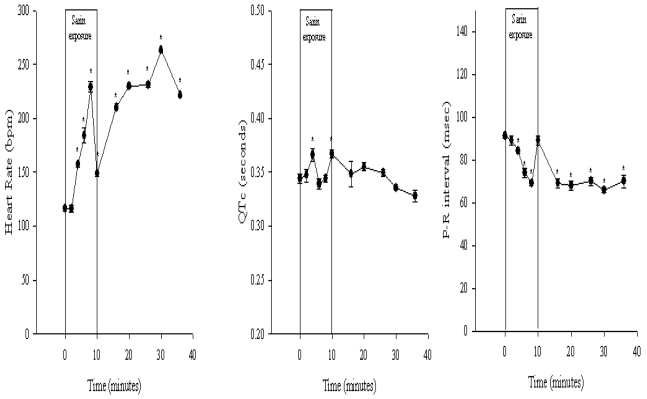


Figure 3

3.4 Cardiac changes

Figures 4A, 4B and 4C show changes in heart rate, QTc and P-R interval over the course of the GB exposure. There is a significant increase in heart rate (Figure 4A; left) as early as 4 minutes into agent exposure, likely due to an increase in sympathetic tone. The heart rate remains elevated up to 30 minutes after the end of the exposure (40 minutes total). There are transient increases in the QTc interval at 4 and 10 minutes (Figure 4B; middle), suggestive of repolarization abnormalities in the ventricles. The decrease in the P-R interval at various times (Figure 4C; right) is suggestive of enhanced conduction from the atria to the ventricles. The cardiac recordings were generated using a standard lead II ECG configuration and data was collected with the Bio-logic Inc. physiological monitoring system. Dataquest A.R.T. was used to analyze the raw data and Statview was used to look for statistical differences (*indicates $p<0.05$). Each data point represents an average (±SEM) of 10-20 consecutive cardiac cycles taken at that specific time.



Figures 4A, 4B & 4C.

3.5 EEG changes

Disposable external self-adhesive leads (Nicolet) were attached to Fz, Cz, Oz, A1 and A2. Data was collected continuously (at a rate of 256 points/second) during the GB exposure using the Bio-logic physiological monitoring system. 30-second epochs were selected during multiple time periods and were analyzed for power spectra using Dataquest A.R.T. By approximately 6 minutes into the exposure there are the first signs of EEG (on the A1-A2 montage) changes with increases in power (not shown) as compared to baseline. The most prominent increase in power occurs in the delta frequency (1-3.5 Hz). The increased power becomes most pronounced at 10 minutes and begins to dissipate by 35 minutes.

3.6 EMG

Disposable self-adhesive electrodes were attached to the pig's back and used to collect EMG data on the Bio-logic physiology system before during and after the exposure. Approximately 30-second epochs were selected at various times during (every 2 minutes) and after the GB exposure (every 5 minutes) and were analyzed for power spectra using Dataquest A.R.T. The first noticeable evidence of an increase in power is seen at 20 minutes (not shown) with the dominant power changes occuring in the 66-90 Hz frequency range. This power increase corresponds with the onset of clinical signs of muscle tremors. The clinical tremors and the EMG power spectral changes were still evident at 35 minutes (not shown).

3.7 Blood Chemistry

An iStat portable clinical analyzer was used to monitor blood chemistry for changes in glucose, pH, lactate, sodium, potassium, hematocrit, hemoglobin, TCO_2, PCO_2, SaO_2, and base excess before during and after the GB exposure. There were no significant changes in any of the blood parameters at any time during the exposure (not shown) accept for a decrease in SaO2 (venous) from 80% to 66% corresponding with the respiratory depression (seen in figure 3). There was a trend of an increase in glucose and hematocrit, as compared to baseline values, corresponding with the progression of clinical signs (tremor, miosis, salivation) of nerve agent toxicity however this trend was not significant.

3.8 Tissue Distribution

24-hours after the conclusion of the GB exposure a final blood sample was collected and the pig was

euthanized and perfused with approximately 4-liters of Ringer's solution. Tissue sections were removed, snap-frozen in liquid nitrogen and subsequently analyzed for regenerated GB via the methods of Jakubowski et al (2002). The figure on the left displays results obtained from internal organs and the figure on the right displays results obtained from tissues related to the Central Nervous System. The kidneys and lungs contained the largest amounts of GB with the lowest amounts being found in the dermis, adipose and muscle (trapezius). Note the different Y-axes scales on the left and right figures.

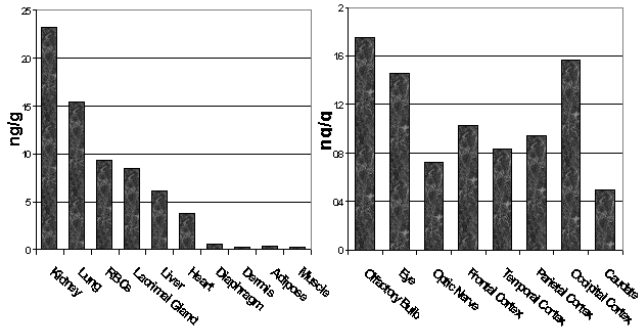


Figure 7

4. CONCLUSIONS

The first noticeable sign of GB intoxication is traditionally considered to be constriction of the pupil. Indeed, in this study the pig had the onset of miosis (10%) by approximately 41/2 minutes into the exposure. The onset and progression of pupil constriction roughly corresponded with changes in heart rate, QTc and P-R interval. These changes are most likely reflective of the onset of sympathetic tone dominance and the beginning of the fight-or flight response.

The first evidence of respiratory depression was not seen until the entire exposure had been completed (10 minutes) and the systemic GB had peaked. The respiratory depression had progressed to its most severe level by 20 minutes. The respiratory depression in our studies was characterized by a decrease in respiratory rate with a corresponding increase in the area under the curve per breath. In laymen's terms the pigs breathing became slow and irregular but the breaths were longer and deeper. The respiratory depression evidenced by the decrease in respiratory rate is consistent with work done prior in pigs using intravenous GB (Duncan, et al., 2001). Duncan and his colleagues also found that there was an increase in tidal volume in conjunction with the decreased respiratory rate. This compensatory response resulted in no net change in the minute volume. There was also evidence of increased airway resistance. Howeve

While the first evidence of overt clinical signs were not seen until approximately 41/2 minutes into the exposure there were detectable (measurable) levels of GB in the RBCs (and to a much lesser extent in the plasma) by 2 minutes into the exposure. Concordantly, the RBC cholinesterase activity was decreased significantly. Given that the first blood sample was drawn at 2 minutes it is safe to say that we failed to detect the earliest possible indication of GB in the blood due to the time of the blood draw. Future experiments will attempt to define the first evidence of systemic GB circulation by extracting blood samples at earlier time points. Interestingly, plasma cholinesterase activity remained unchanged for the duration of the exposure. The pig, like the human, has no circulating carboxylesterase, which may account for this finding.

The estimated lethal concentration for a 10-minute vapor exposure to GB in male swine is 6.90 mg/m^3. The dosage of vapor GB in this study (LCt_{50}=53.5 mg-min/m^3) was considered to be an exposure that would cause mild to moderate signs of organophosphate toxicity. The plethora of signs elicited by exposure to acetylcholinesterase inhibitors has been widely addressed (Holstege et al., 1997) and it comes as no surprise that the pig in these studies quickly developed miosis, tremors, ECG and EEG irregularities. The results presented herein are not intended to portray that every inhalation exposure to vapor GB will elicit the same time-course or severity of toxic signs but rather to reveal that we have the capabilities to monitor the effects of such an exposure on multiple systems in real time. The ability to monitor the time course of these signs in relation to the amount of sarin in the systemic circulation and the eventual tissue distribution is what sets this study apart from others.

5. TIMELINE SUMMARY

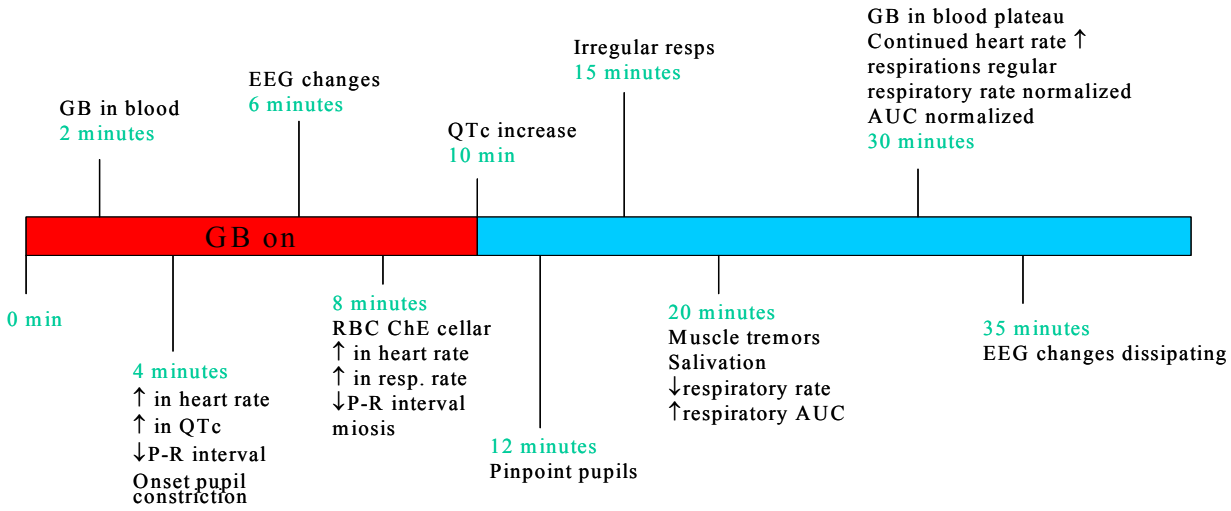


Figure 8. Timeline of toxic changes elicited by a 10-minute inhalation exposure to 5.35 mg/m^3 vapor GB in a male, sexually-mature Gottingen minipig.

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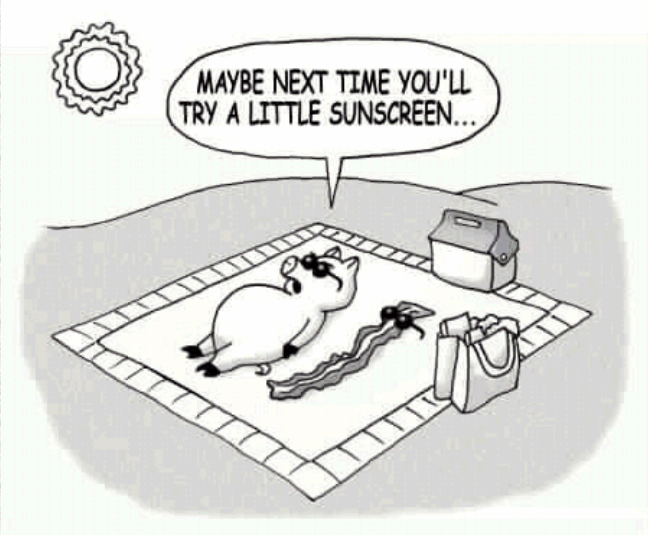
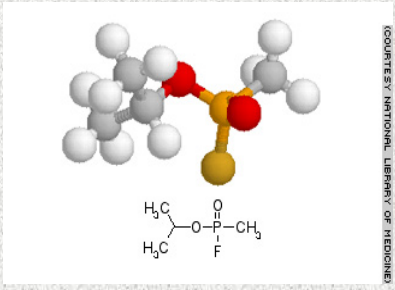


“Whether or not gas will be employed in future wars is a matter of conjecture, but the effect is so deadly to the unprepared that we can never afford to neglect the question.”

Final Report of General John J. Pershing. Washington, DC: US Government Printing office; 1920:77

Nerve Agents

- Organophosphate cholinesterase inhibitor
- S.L.U.D.
- Eye
  - Miosis, pain
- Pulmonary
  - Bronchoconstriction, tight chest, cough
- Muscular
  - Fasciculations, flaccid paralysis
- GI
  - Nausea, vomiting, abdominal cramps
- Skin
- Cardiovascular
- CNS
  - Loss of consciousness, convulsions, apnea



(COURTESY NATIONAL LIBRARY OF MEDICINE)

- Sarin
- German Agent B (GB)

- Tabun (GA), Soman (GD), Cyclo-sarin (GF), VX

Sarin Use

- Iran-Iraq war
- Khamisiyah
- Tokyo Subway
  - Sarin placed in 5 subway cars
  - 12 dead
  - >5000 injured

“Gas is insidious. It often causes casualties without any warning. It exerts a tremendous effect on morale, especially in untrained troops. Uncertainty as to when and where gas is present and how it will act is demoralizing even to troops with high discipline. Nothing breaks a soldier’s will to fight so quickly as being gassed, even slightly. His imagination magnifies his real injury 100-fold.”

Frederic Brown – taken from *Chemical Warfare: a Study in Restraints*

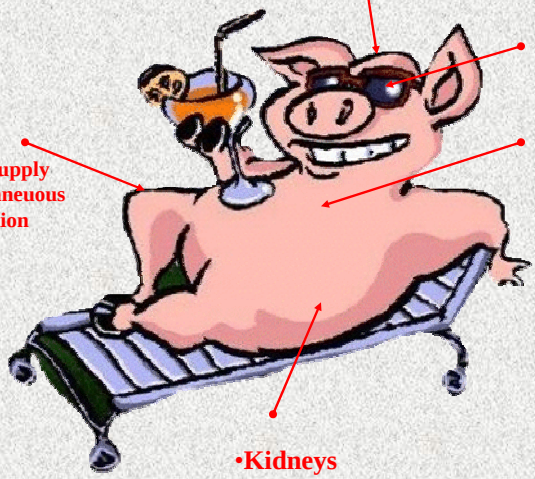
Operationally Relevant Questions

- How sensitive do detectors need to be?
- Do masks and protective clothing afford complete protection?
- When is it safe to come out of protective posture?
- How dirty is clean?
- Are we using the best medical countermeasures?

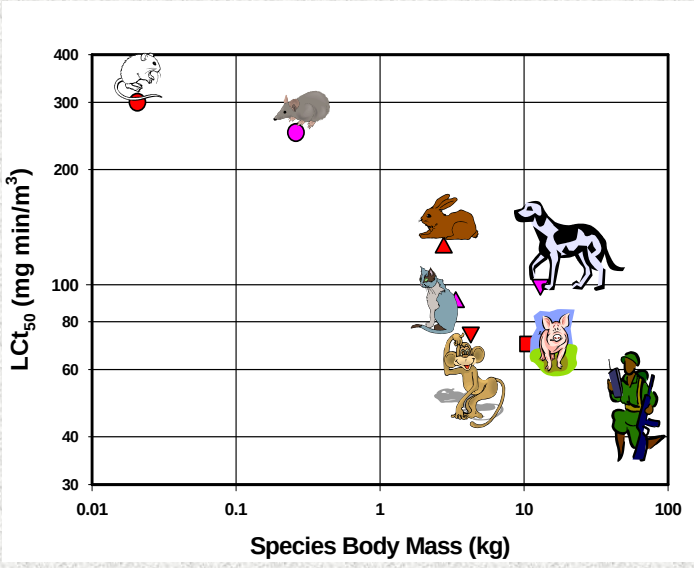
Challenges in Nerve Agent Research

- Route of Entry (sc, iv, im, ip, pc, ih)
- Inhalation exposures
  - Generation of consistent nerve agent concentrations
  - Accuracy and precision of nerve agent concentration measurements
  - Logistics and safety issues with collecting data DURING a real-time exposure
    - Historically data was collected "after-the-fact"
- What is the most appropriate animal model to extrapolate to humans?

Benefits of the minipig



Body Mass/LCt_{50} Relationship for GB (10-Minute Exposure)



LCT_{50} = Conc. x Time (mg. min/m^3) for lethality in 50% of the exposed population

Gottingen Minipigs

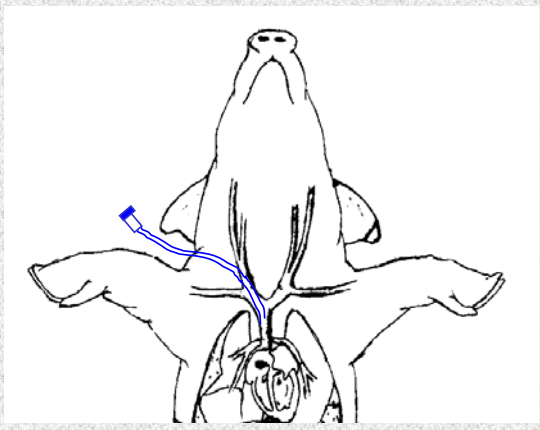
- Well defined genetic background and health status
- Small size at sexual maturity
  - Males 7-9 kg (3-4 months)
  - Females 9-11 kg (4-5 months)

"I like pigs. Dogs look up to us. Cats look down on us. Pigs treat us as equals."

-Winston Churchill

Experimental Preparations

- Gottingen (Ellegaard) minipigs (10-15 Kg)
  - Pigs surgically prepared with external jugular cannulas
    - Allows serial blood sampling during exposure



GB Vapor Exposure chamber

- Pig placed in sling
- Electrophysiology leads attached to pig and leading to Bio-logic headbox.
- 1000 L dynamic airflow chamber
- GB generation system contained in glove box
  - Vapor Sampling / Analysis
- Jugular catheter passed through ports

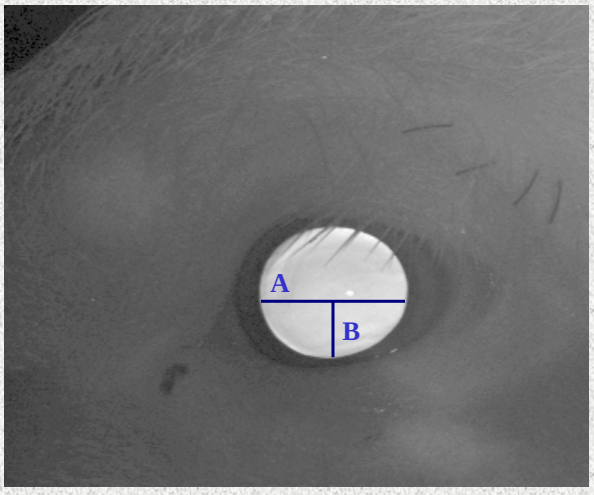
- 5.35 mg/m^3 vapor GB for 10 minutes (Ct=53.5 mg.min/m^3)

Data collection methods / Biological endpoints

- Infrared camera
  - Allows pupil images under dim light conditions
  - Uses pupil areas to plot time-to-miosis
- Insertion of external jugular catheter
  - Serial blood samples during “real-time” exposure
    - RBC and plasma cholinesterase
    - GB regeneration assay
    - Clinical Blood analysis
- Physiological monitoring (Bio-logic, Inc.)
  - ECG, respiratory rate, EEG, EMG, airflow, eye movements, SaO_2
- Necropsy
  - Tissue GB levels
  - Changes in gene transcription

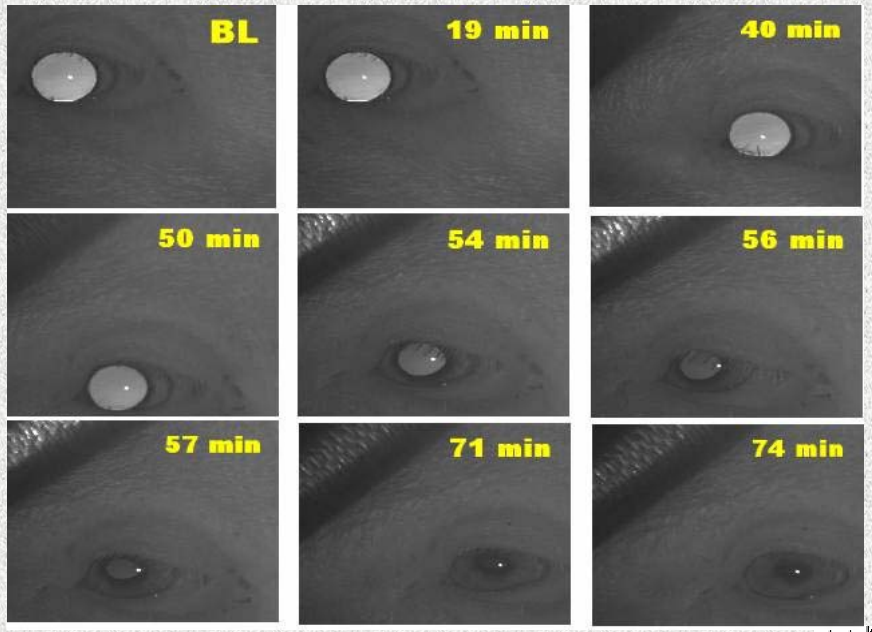
Infrared pupillometry

Baseline

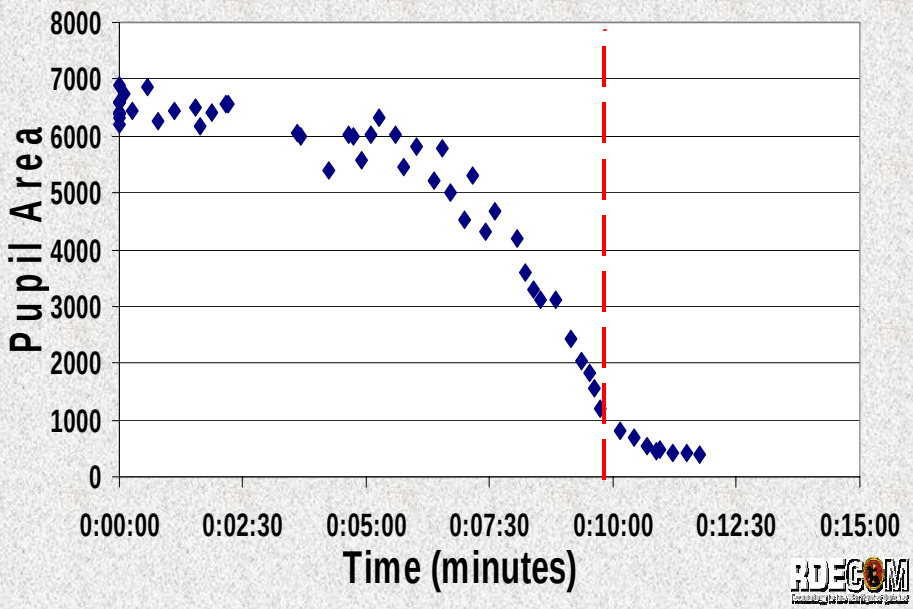


- Infrared light reflects off of the retina
- Pupil area = A*B*π

Example of GB Induced Pupil Size Changes Over Time



Effect of GB Vapor on Pupil Constriction



Data collection methods / Biological endpoints

- Infrared camera
  - Allows images under dim light conditions
  - Uses pupil areas to plot time-to-miosis
- Insertion of external jugular catheter
  - Serial blood samples during "real-time" exposure
    - RBC and plasma cholinesterase
    - GB regeneration assay
    - Clinical Blood analysis
- Physiological monitoring (Bio-logic, Inc.)
  - ECG, respiratory rate, EEG, EMG, airflow, eye movements, SaO_2
- Necropsy
  - Tissue GB levels
  - Changes in gene transcription

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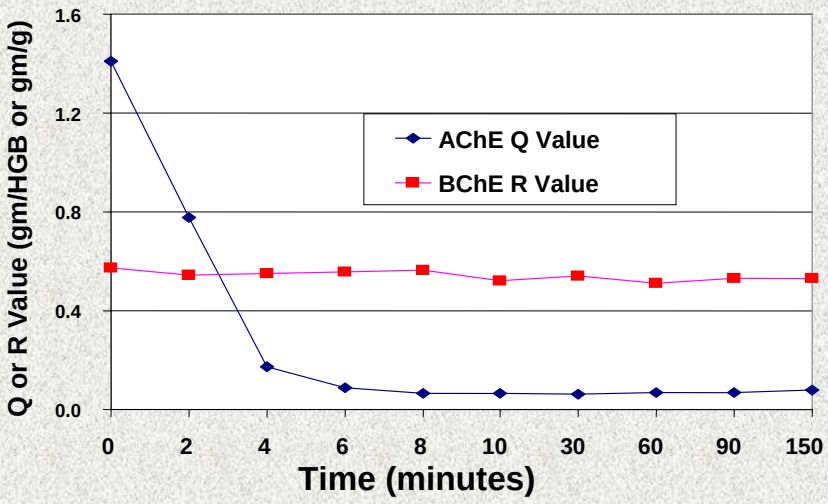
Effect of GB Vapor on Cholinesterase Activity



Data collection methods / Biological endpoints

- Infrared camera
  - Allows images under dim light conditions
  - Uses pupil areas to plot time-to-miosis
- Insertion of external jugular catheter
  - Serial blood samples during “real-time” exposure
    - RBC and plasma cholinesterase
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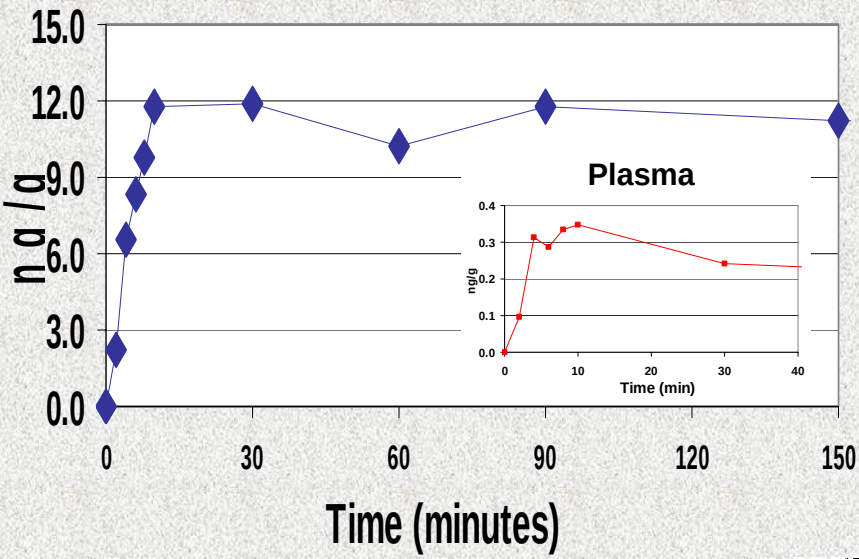
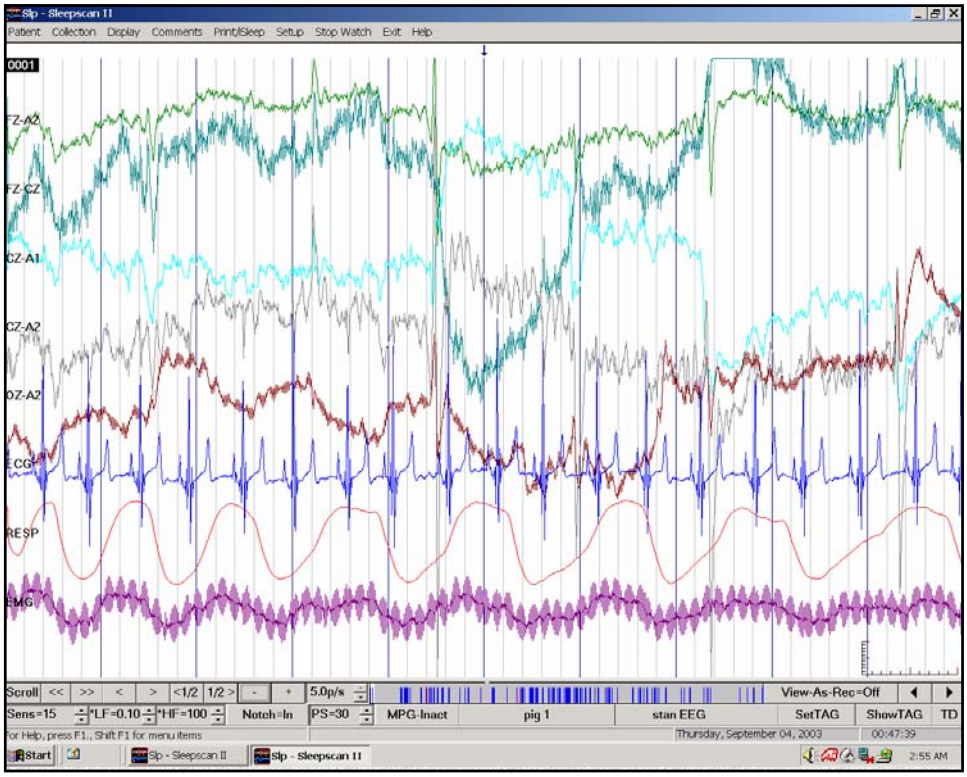
  - ECG, respiratory rate, EEG, EMG, airflow, eye movements, SaO_2
- Necropsy
  - Tissue GB levels
  - Changes in gene transcription

Effect of GB Vapor on Regenerated GB



Data collection methods / Biological endpoints

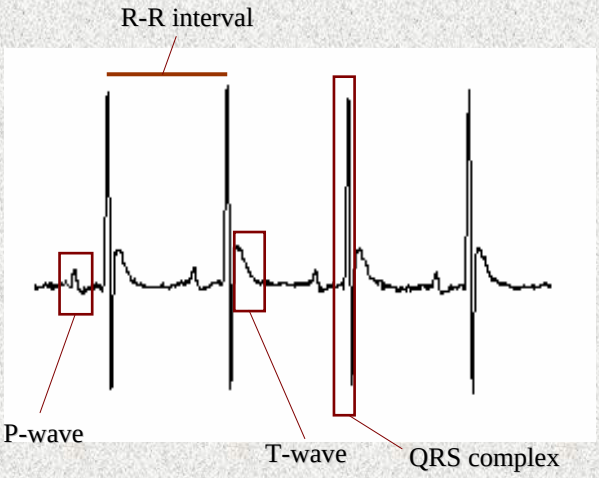
- Infrared camera
  - Allows images under dim light conditions
  - Uses pupil areas to plot time-to-miosis
- Insertion of external jugular catheter
  - Serial blood samples during “real-time” exposure
    - RBC and plasma cholinesterase
    - GB regeneration assay
    - Clinical Blood Analysis
- Physiological monitoring (Bio-logic, Inc.)
  - ECG, respiratory rate, EEG, EMG, airflow, eye movements, SaO_2
- Necropsy
  - Tissue GB levels
  - Changes in gene transcription



ECG analysis

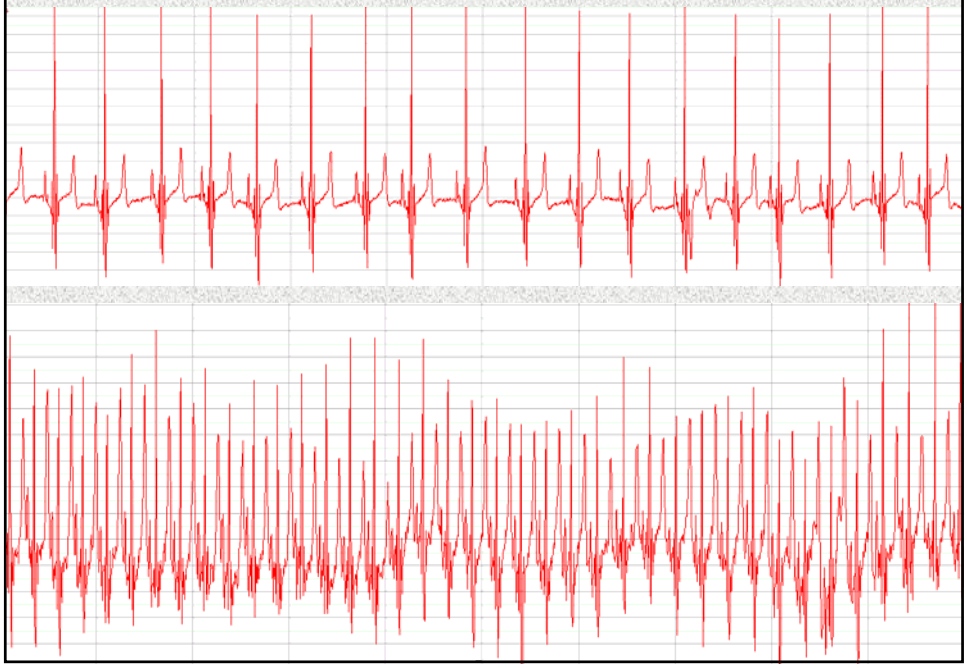
- ECG signals recorded on Biologic Inc. system
- Standard Lead II configuration
  - Historical data available
- Exposure broken down into 2 minute segments
  - Approximately 30 second epochs during each segment analyzed
- Data analyzed using Dataquest ART and statview
  - Heart rate (R-R interval)
  - PR interval
  - QT interval
  - QTc interval
  - ST interval
  - QRS interval

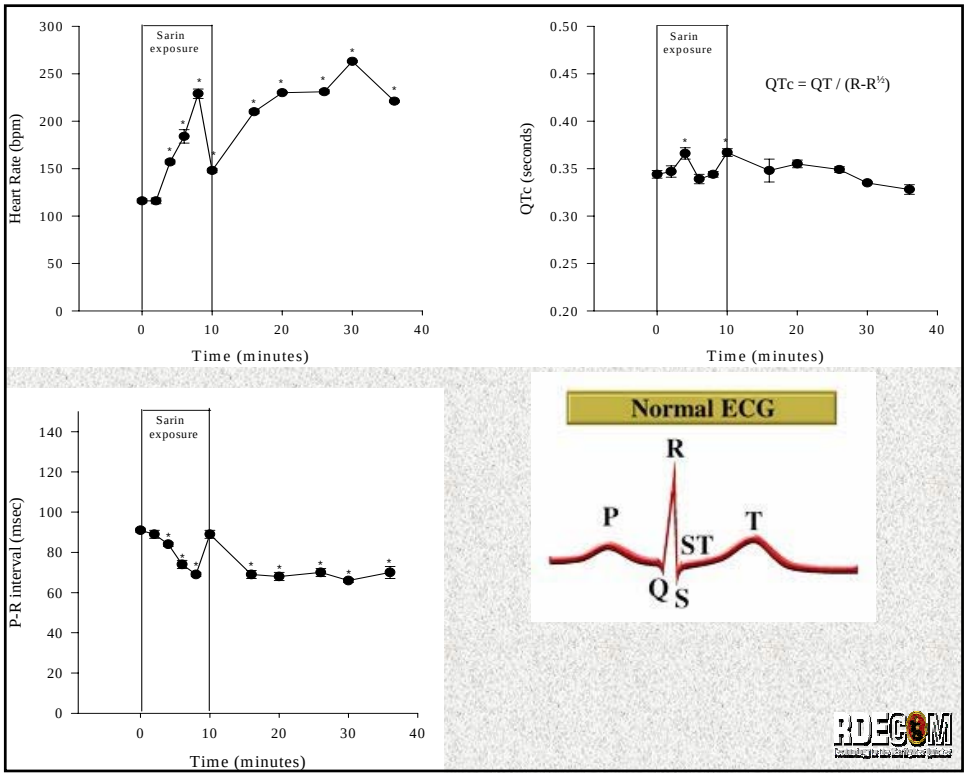
Effect of GB vapor on the ECG



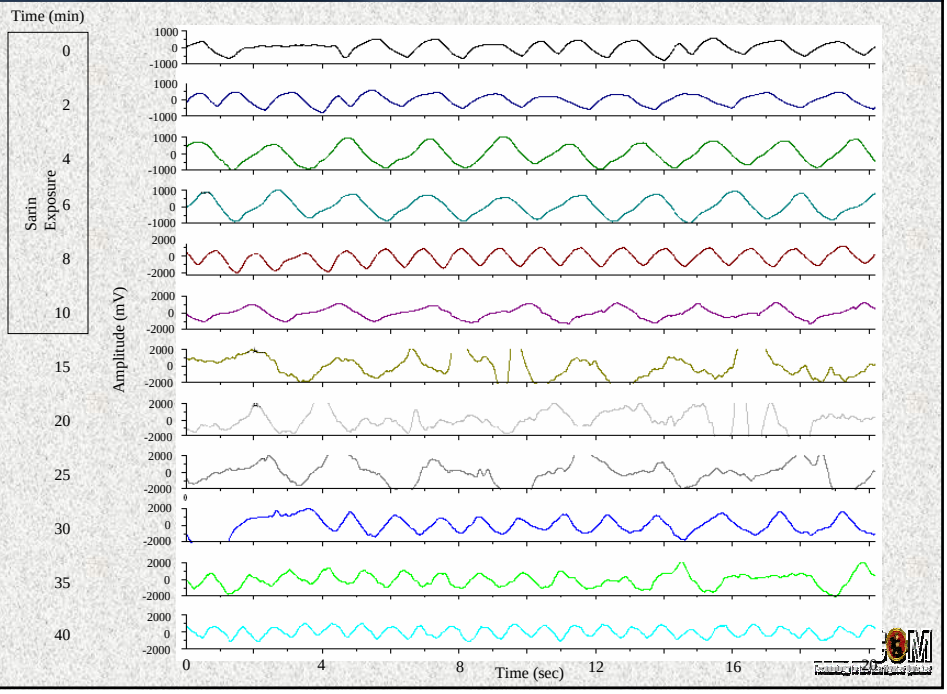
- R-R interval – time between successive ventricular beats
- QRS complex – ventricular contraction
- T-wave – ventricular repolarization
- QT segment – time from ventricular contraction to the end of ventricular repolarization
- PR segment – time for atrial depolarization to enter the ventricles via the AV node

Effect of GB Vapor on ECG

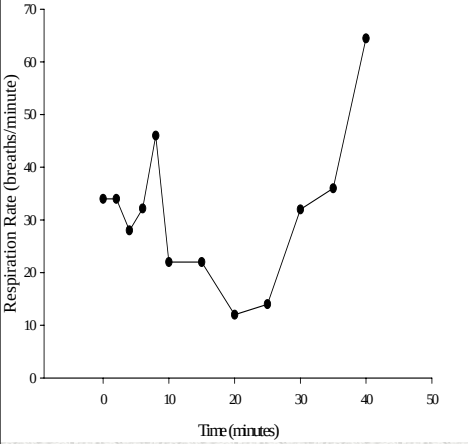


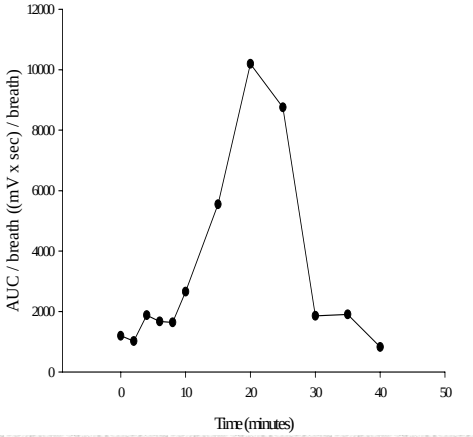


Effect of GB Vapor on Respiratory Traces



Effect of GB Vapor on Respiratory Rate and AUC

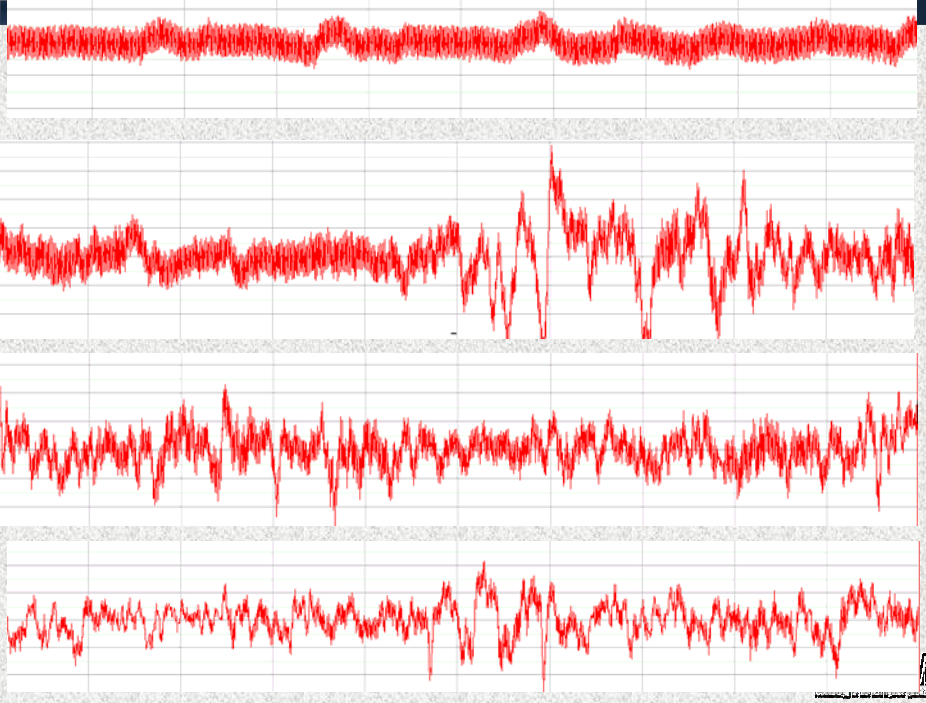




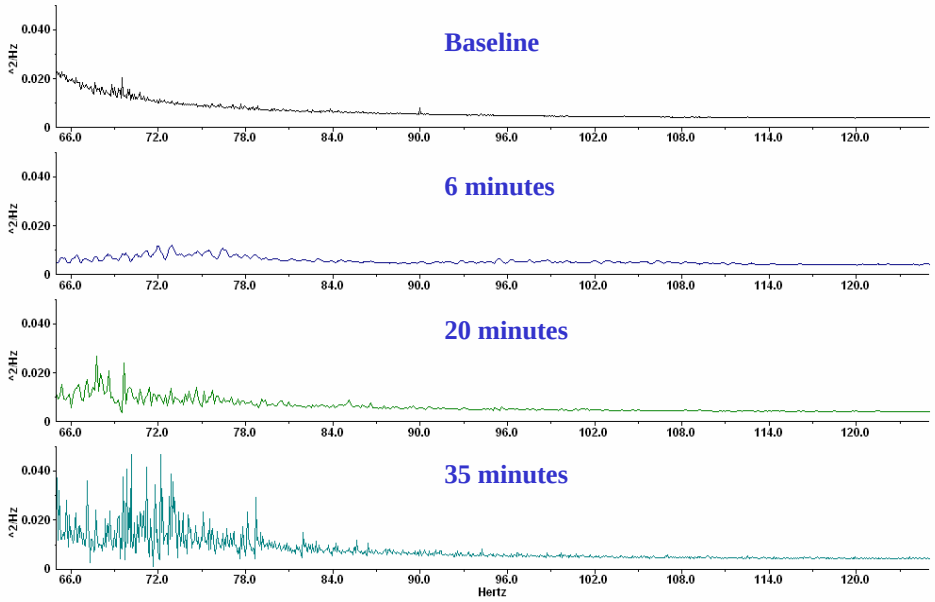
EMG analysis

- EMG signals recorded on Biologic Inc. system
- Exposure broken down into 2 minute segments
  - Approximately 30 second epochs during each segment analyzed
- Power spectral density analysis using Persyst.

EMG

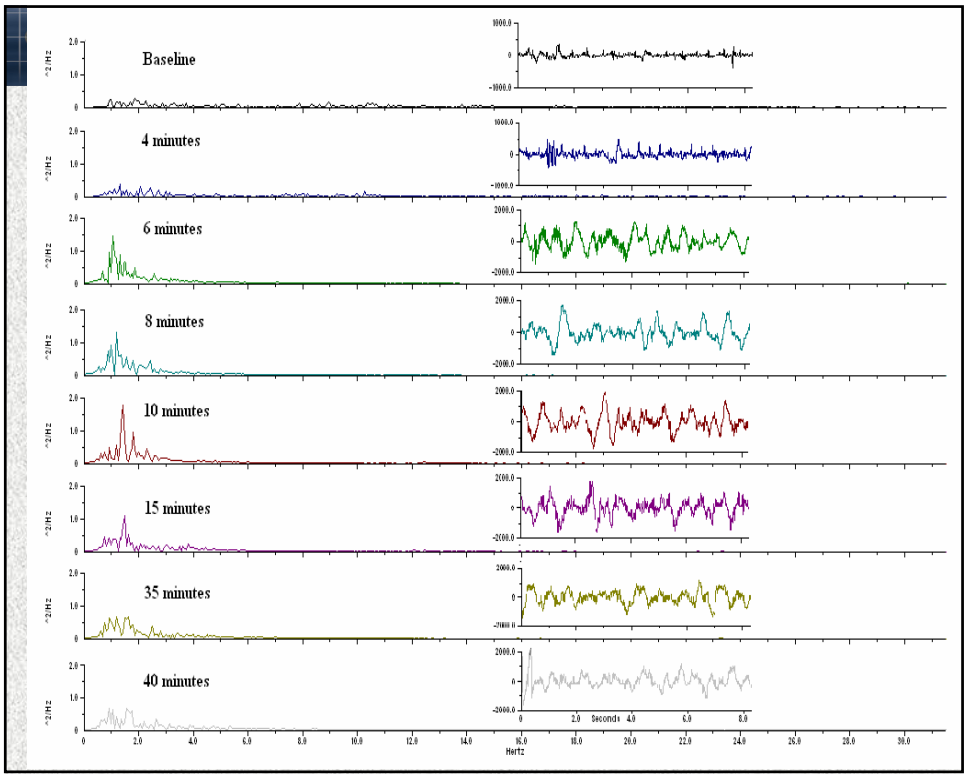


EMG power spectral analysis



EEG analysis

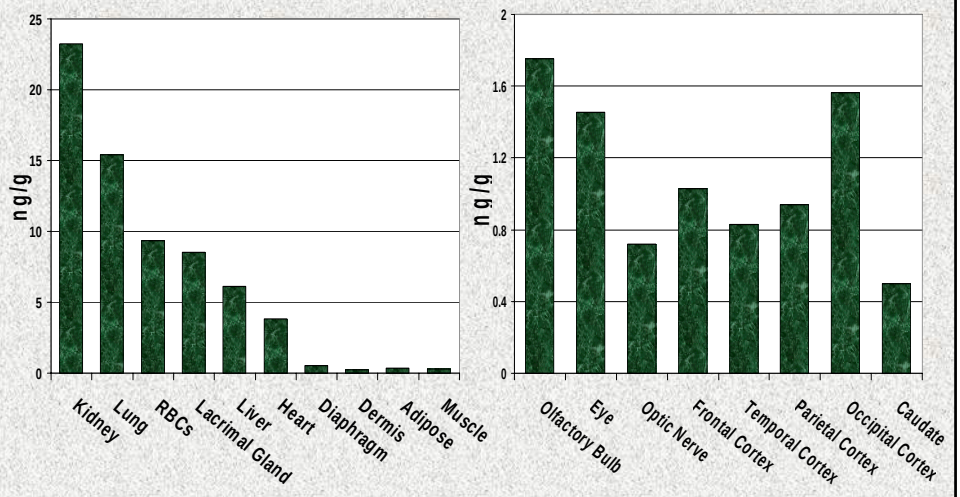
- EEG signals recorded on Biologic Inc. system
- Exposure broken down into 2 minute segments
  - Approximately 30 second epochs during each segment analyzed
- Power spectral density analysis using Persyst.
  - Bandpass filters: 1-35 Hz



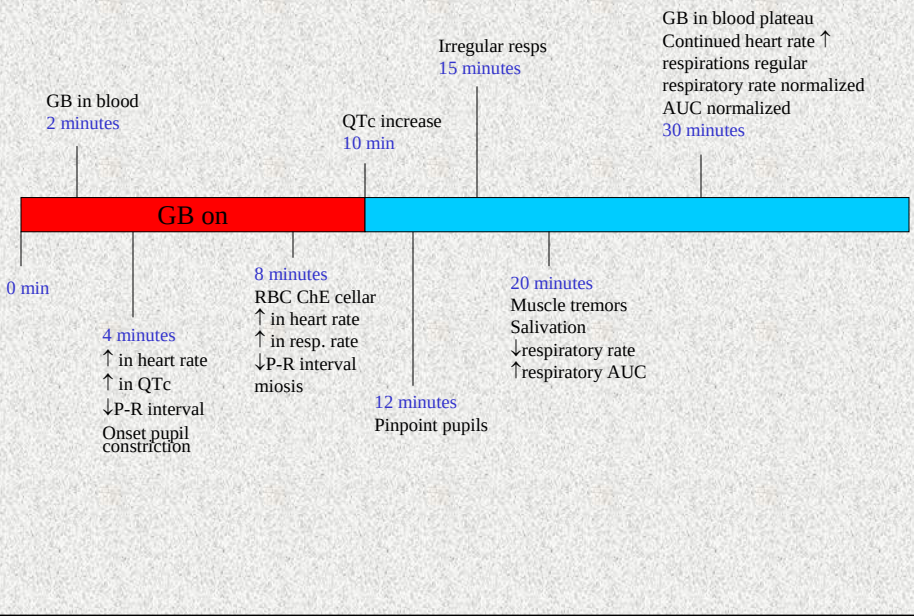
Data collection methods / Biological endpoints

- **Infrared camera**
  - **Allows images under dim light conditions**
  - **Uses pupil areas to plot time-to-miosis**
  - **Calculation of EC_{50} (miosis)**
- **Insertion of external jugular catheter**
  - **Serial blood samples during "real-time" exposure**
    - **RBC and plasma cholinesterase**
    - **GB regeneration assay**
    - **Clinical blood analysis**
- **Physiological monitoring (Bio-logic, Inc.)**
  - **ECG, respiratory rate, EEG, EMG, airflow, eye movements, SaO_2**
- **Necropsy**
  - **Tissue GB levels**
  - **Changes in gene transcription**

Effect of GB Vapor on Regenerated GB in Tissue



SUMMARY: Time Course of Toxic Effects of GB Vapor Exposure



CONCLUSIONS

External Concentration

↓

Internal Concentration

↓

Signs of exposure

↓

Predictive Modeling/PBPK

↓

Exposure Guidelines

When to mask/ unmask?

Is equipment still contaminated?

detectors sensitive enough?

Thank You

- E. Mike Jakubowski
- Paul Dabisch
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- Jill Jarvis
- Dennis Miller
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- Ruth Way
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- James Manthei
- Jennifer Edwards
- Jeff McGuire
- Ron Evans
- Bernardita Gaviola
- David McCaskey
- Ron Crosier
- Melvin Ware
- Dennis Johnson

Low Level Operational Toxicology

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LCt_{50} vs. Time

Haber's Rule (CT=k)

Toxicity is directly related to concentration integrated over time (i.e., C x T, CT, or dosage) (Haber, 1924)

Toxic Load Model ($C^nT=k$)

Concentration has more effect than time